Supplemental data

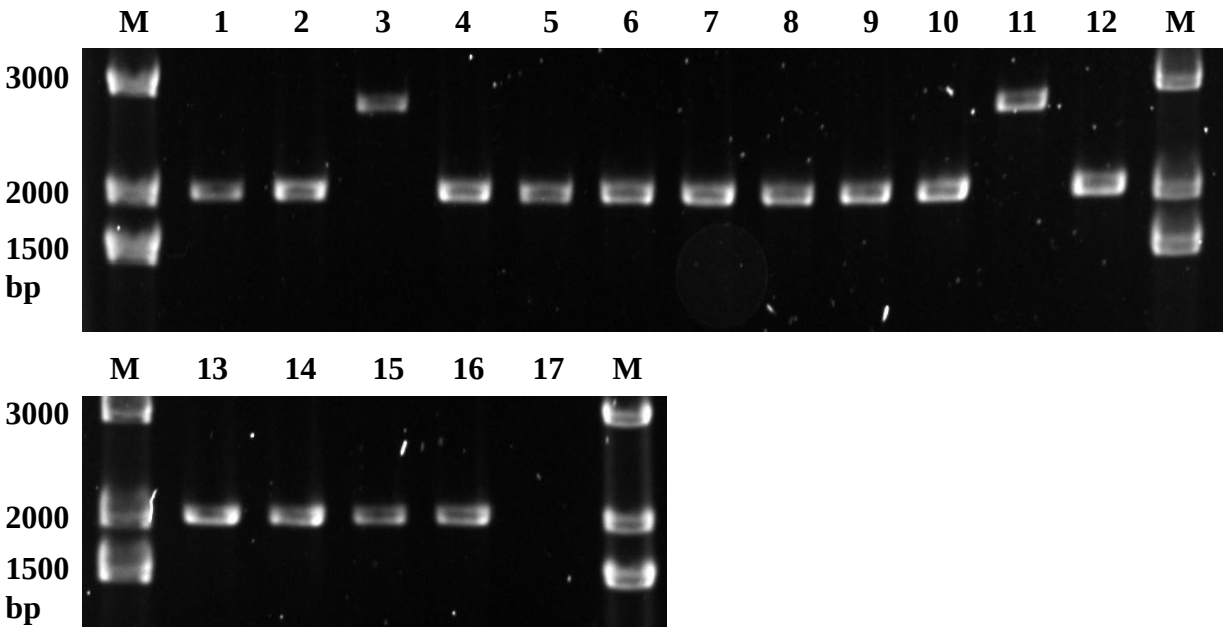
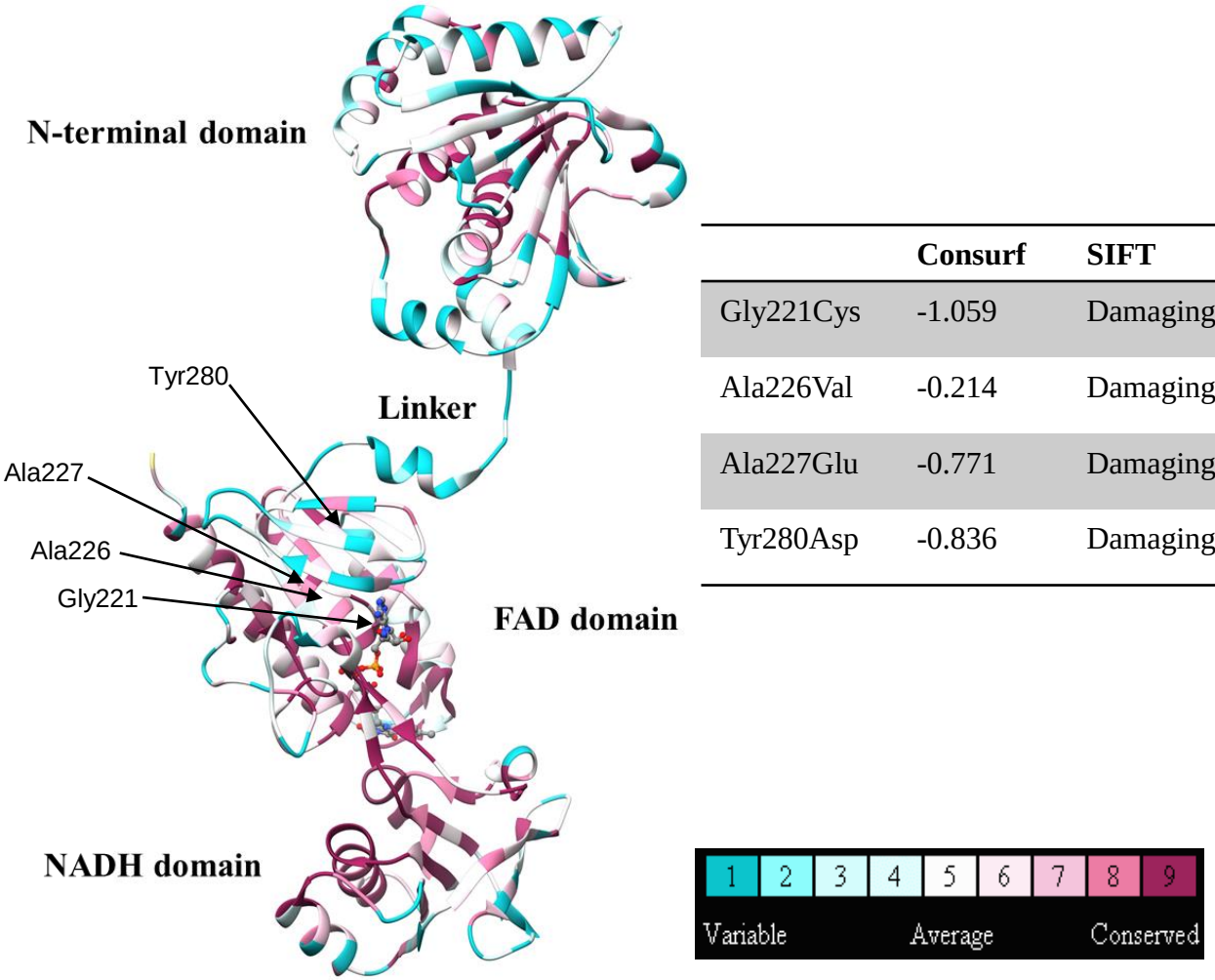


Figure S1: The PCR products of the *ahpF* gene derived from FZ-resistant mutants. Lanes M, 1 kb plus ladder; 1, FZ08; 2, FZ10; 3, FZ11; 4, FZ12; 5, FZ13; 6, FZ14; 7, FZ15; 8, FZ16; 9, FZ17; 10, FZ18; 11, FZ19; 12, FZ20; 13, FZ21; 14, FZ22; 15, FZ23; 16, parental strain K2479; 17, non-template control. The size of *ahpF* amplicon in two mutants FZ11 and FZ19 was larger than that of the parental strain by 800 bp, indicating an 800-nucleotide insertion within the *ahpF* gene in these two FZ-resistant mutants.



	Consurf	SIFT
Gly221Cys	-1.059	Damaging
Ala226Val	-0.214	Damaging
Ala227Glu	-0.771	Damaging
Tyr280Asp	-0.836	Damaging

Figure S2: Evolutionary conservation analysis of AhpF (PDB ID 4O5Q). The evolutionary conservations of the AhpF amino acid sequence were analyzed using the Consurf web server and the effect of single amino acid substitutions on protein function (damaging or non-damaging) was predicted using the SIFT web server. The table on the right shows the normalized conservation score of protein residues which were mutated in FZ-resistant mutants. The 3D backbone of AhpF was colored according to the color-scaled conservation score of its residues calculated by the Consurf web server.

The residues Gly221, Ala226, Ala227 and Tyr280 had negative normalized conservation scores, indicating that these residues are highly conserved during the course of evolution. Mutations in these residues are highly likely to cause a loss of protein structure and function. Similarly, all the four missense mutations, including Gly221Cys, Ala226Val, Ala227Glu and Tyr280Asp were predicted by SIFT to cause the damaging effect to the AhpF protein with high confidence.

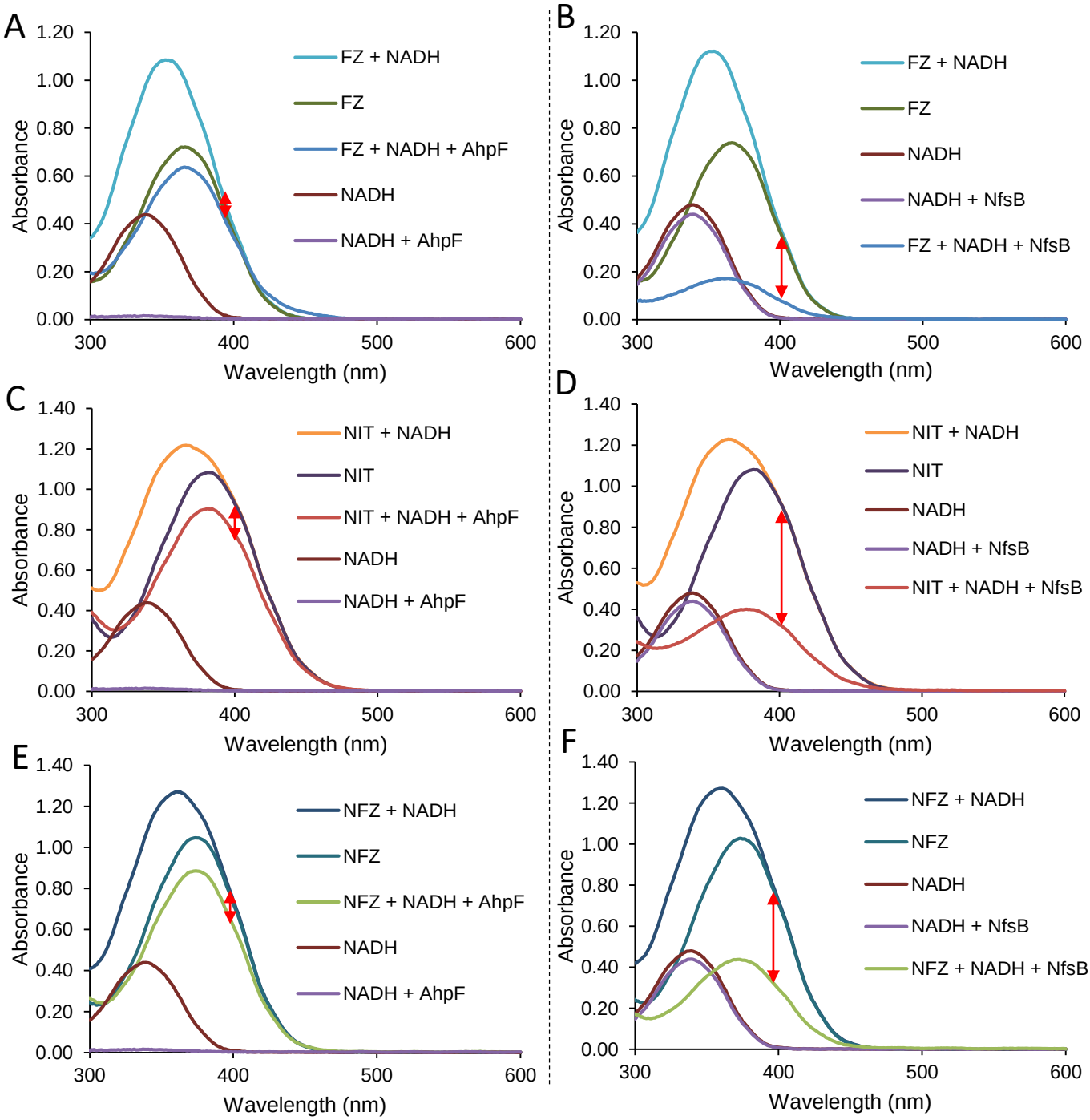


Figure S3: *In vitro* 5-nitrofuran reduction by AhpF and NfsB under aerobic conditions. The absorbance spectrum (300 - 600 nm) of each reaction was measured at the end of the assay (12 h). Purified AhpF 5 µg/mL (A, C, E) or NfsB 1 µg/mL (B, D, F) was mixed with furazolidone (FZ; A, B), nitrofurantoin (NIT; C, D) or nitrofurazone (NFZ; E, F) and NADH at the ratio of 0.1 mM : 0.1 mM. Each data point represents the mean of three replicates. No-enzyme control and the substrates alone were included to allow comparison of the absorbance spectra before and after the reaction. Red arrows indicate the difference in the absorbance due to the nitrofuran reduction.

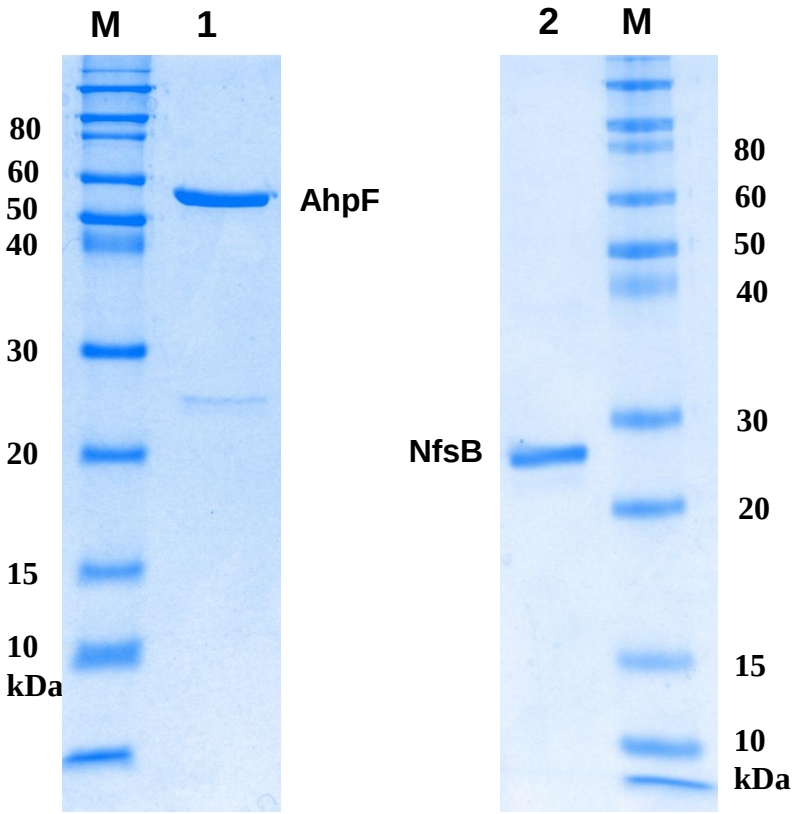


Figure S4: Purification of AhpF and NfsB. SDS-PAGE analysis of the His-tagged AhpF and and the His-tagged NfsB purifcations. Lanes: M, Novex sharp pre-stained protein standard (Invitrogen^{TM}); 1, AhpF purified sample; 2, NfsB purified sample. Ni-NTA affinity purification of AhpF (57 kDa) resulted in about 90.3 % purity with one contaminant protein band accounting for the remaining 9.7 %. No non-specific bands were observed in the purified NfsB sample.